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Abstract: We examined how HLA types A1-B8-DR3 and B27 were related to progression of clinical disease and rate of loss of CD4 lymphocytes in the Edinburgh City Hospital cohort of HIV-positive patients, mainly injection drug users. Patients ($n = 692$) were prospectively followed from 1985 through March 1994. Accurately estimated seroconversion times were determined retrospectively for a subgroup of 313 (45%). Of 262 patients (39%) who were fully or partially HLA typed, 155 (50%) had known seroconversions. Of 34 patients typed positive for A1-B8-DR3, 29 progressed to CDC stage IV, 22 to AIDS and 20 died. Twelve patients were typed positive for B27; six of these progressed to CDC stage IV, one to AIDS and none died. In a proportional hazards analysis of the 313 patients with known seroconversions, A1-B8-DR3 was significantly associated with covariate-adjusted relative risks of 3.7 (95% CI 1.9-7.2), 3.1 (1.6-6.0) and 1.9 (1.1-3.2) for progression from seroconversion to death, AIDS and CDC stage IV, respectively. Events for B27 were too rare to include B27 in analyses to death and AIDS, but B27 was significantly associated with slower progression to CDC stage IV (0.3, CI 0.1-0.9). Random effects growth curve models were used to estimate individual rates of loss of square root CD4 count and loss of CD4 percentage, for 603 and 617 patients, respectively. A1-B8-DR3 was associated with rapid loss of both markers ($p=0.02$ and $p = 0.01$, respectively); B27 was associated with slow loss of both markers ($p=0.04$ and $p<0.005$)

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Original Papers

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Association of HLA types A1-B8-DR3 and B27 with rapid and slow progression of HIV disease

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Summary

We examined how HLA types A1-B8-DR3 and B27 were related to progression of clinical disease and rate of loss of CD4 lymphocytes in the Edinburgh City Hospital cohort of HIV-positive patients, mainly injection drug users. Patients ($n=692$) were prospectively followed from 1985 through March 1994. Accurately estimated seroconversion times were determined retrospectively for a subgroup of 313 (45%). Of 262 patients (39%) who were fully or partially HLA typed, 155 (50%) had known seroconversions. Of 34 patients typed positive for A1-B8-DR3, 29 progressed to CDC stage IV, 22 to AIDS and 20 died. Twelve patients were typed positive for B27; six of these progressed to CDC stage IV, one to AIDS and none died. In a proportional hazards analysis of the 313 patients with known seroconversions, A1-B8-DR3 was significantly asso-

ciated with covariate-adjusted relative risks of 3.7 (95% CI 1.9–7.2), 3.1 (1.6–6.0) and 1.9 (1.1–3.2) for progression from seroconversion to death, AIDS and CDC stage IV, respectively. Events for B27 were too rare to include B27 in analyses to death and AIDS, but B27 was significantly associated with slower progression to CDC stage IV (0.3, CI 0.1–0.9). Random effects growth curve models were used to estimate individual rates of loss of square root CD4 count and loss of CD4 percentage, for 603 and 617 patients, respectively. A1-B8-DR3 was associated with rapid loss of both markers ($p=0.02$ and $p=0.01$, respectively); B27 was associated with slow loss of both markers ($p=0.04$ and $p<0.005$).

Introduction

Several studies have reported associations between HIV disease progression and HLA antigens. Steel *et al.*¹ investigated 32 Edinburgh haemophilia patients exposed to a single batch of blood factor contaminated with HIV, of whom 18 became HIV-antibody-positive. They found that the antigen combination A1-B8-DR3 (usually identified as a haplo-

type) was weakly associated with an increased risk of seroconversion (relative risk 2.9) while, in those who seroconverted, it was strongly associated with the development of HIV-related symptoms; of 11 patients who developed symptoms, eight possessed the haplotype, whilst none of seven patients remaining symptom-free possessed the haplotype

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(relative risk 28). Graphical evidence that A1-B8-DR3 patients experienced faster CD4 loss was also noted.

Kaslow *et al.*² provided further confirmation of this finding in a study of 108 seropositive homosexual men, composed of a group of 49 who showed rapid decline in CD4 counts over a two-year period and a group of 59 who showed little or no decline. Rapid decliners were patients with CD4 count and CD4 percentage slopes in the fastest 15% of the Multicenter AIDS Cohort Study (MACS) whilst slow decliners had slopes in the slowest 35%. The groups were matched for study centre, ethnic group and initial CD4 count, and A1-B8-DR3 was significantly more common in the rapid progressors (odds ratio 3.8).

Kaplan *et al.* found the A1-B8-DR3 and B8-DR3 combinations to be significantly associated with progression from presentation to AIDS in 180 seropositive Europeans³ (relative risks 7.6 and 10.6 from Cox model). They also found these combinations to be significantly associated with rates of six-month CD4 lymphocyte loss greater than 20% (RRs 2.7 and 2.2).

We have followed up this work by investigating the influence of HLA type A1-B8-DR3 on progression and rate of loss of CD4 in a large cohort of HIV-infected patients where, for a large subset of the cohort, a narrow interval estimate of the date of seroconversion was available. We also investigated possible associations with B27, because of its known association with other diseases (ankylosing spondylitis, Reiter's syndrome) and, more importantly, because of recent work suggesting there are similarities between HLA class 2 DR B1 chain and the carboxyterminus of the HIV-1 envelope gp120, when presented as peptides by B27, which leads to the hypothesis that B27 might be associated with slower progression.⁴ An earlier analysis of the Edinburgh data⁵ gave credibility to this hypothesis.

Data are from the Edinburgh City Hospital Cohort,⁶⁻¹⁰ a prospectively followed cohort which commenced recruitment in 1985, and which is composed of HIV-positive patients who visited the Infectious Diseases Unit of the City Hospital from 1985 onwards; the modal risk behaviour in the cohort is injection drug use. Because of a hepatitis B study among Edinburgh injection drug users (IDUs) in the early 1980s, a large amount of stored sera is available for Edinburgh IDUs which has made it possible to establish retrospectively a sub-cohort of patients with well-determined lengths of infection, generally acquired in 1983-85 and mostly through drug use.

Methods

Patients

At 24 March 1994, the Edinburgh City Hospital cohort included 692 patients, of whom 262 (39%)

had been HLA typed. Of these 692, 313 (45%) had narrow interval estimates of seroconversion, and we refer to this subgroup as the seroconversion cohort; 155 members (50%) of the seroconversion cohort were HLA-typed (Table 1).

At 24 March 1994, the cohort contained 206 women (30%) and 470 (68%) injection drug users (IDUs). There had been 221 AIDS cases and 212 deaths (145 AIDS, 26 overdoses, 24 medical non-AIDS, 16 other); 430 patients (62%) had been treated with zidovudine (AZT).

The seroconversion cohort contained 102 women (32%) and was dominated by IDUs (281, 90%); mean age at seroconversion was 23.6 (90% age range 17.5-34.7) and the majority of patients (85%) were estimated to have seroconverted in 1983-85. There had been 84 AIDS cases and 82 deaths (46 AIDS, 13 overdoses, 16 medical non-AIDS, six other); 206 people (66%) had been treated with AZT.

Seroconversion determination

For the seroconversion cohort, seroconversion times were determined by either (i) observation of seroconversion illness, (ii) the midpoint of positive and negative HIV tests not more than two years apart, (iii) the midpoint of January 1983 and a positive HIV test not more than two years after January 1983, the assumed epidemic start date in Edinburgh IDUs, or (iv) detailed knowledge of injection behaviour. Numbers of determinations by the four methods were 13, 99, 166 and 35. Interval estimates by methods (ii) and (iii) were considered to be subject to a year's error at most.

January 1983 was taken as the estimated epidemic start, as it was the earliest known date for any drug user in Edinburgh to have seroconverted for HIV.¹¹ In proportional hazards analyses of clinical progression from estimated seroconversion to clinical endpoints of CDC stage IV, AIDS and death, patients with seroconversions determined from the imputed epidemic start date (iii) had slower progression than patients with seroconversions determined by the other methods; there were no differences between the other methods. When the estimated epidemic start date was temporarily moved forward to July 1983, this effect disappeared. This suggests both that January 1983 is a conservative last negative date in method (iii), and that estimates of seroconversion are accurate enough to reveal such small differences. Analyses of covariates in this paper are controlled for seroconversion determination method.

HLA methodology

The HLA typing exercise was initiated in the sickest patients so that the information could be obtained

Table 1 Summary statistics for whole cohort and seroconversion cohort

Cohort:	Whole		Seroconversion	
Number of patients	692		313	
Number HLA typed	262	39%	155	50%
Infected through IDU	470	68%	281	90%
Homosexually infected	107	15%	10	3%
Heterosexually infected	103	15%	19	6%
Infected through blood products	8	1%	3	1%
Unclear risk activity	4	0%	0	0%
Women	206	30%	102	32%
IDUs who are women (as % of IDUs)	137	29%	88	31%
Heterosexually infected women	67	65%	13	68%
Mean age at enrolment/serocon.* (SD)	29.4	7.5	23.6	5.2
CDC stage IV cases	413	60%	185	59%
AIDS cases	221	32%	84	27%
Deaths:	212	31%	82	26%
AIDS deaths (as % of all deaths)	145	68%	46	56%
Death for medical reasons but not AIDS	24	11%	16	20%
Drugs overdoses	26	12%	13	16%
Unknown causes	16	8%	6	7%
Zidovudine (AZT) recipients	430	62%	206	66%
Mean clinic visits (SD)	19.4	16.1	22.9	15.9

*Enrolment in case of whole cohort; seroconversion for seroconversion cohort

whilst they were still alive, since at the time the study was undertaken it was a much more difficult task to determine HLA types retrospectively from DNA strands in stored sera. Further typing of additional patients then concentrated on gaining more complete information on those with narrow seroconversion intervals. HLA typing was by a standard two-stage complement-dependent microlymphocytotoxicity technique.¹²

Analysis of clinical progression

For the seroconversion cohort, proportional hazards models¹³ from seroconversion to CDC stage IV, AIDS and death were used to explore HLA associations with progression rate. Additional covariates in the models controlled for gender, exposure category, age at seroconversion and method of seroconversion determination; that is, the additional covariates allowed us to estimate HLA effects net of the possible influences of other factors. A covariate for AZT use was not included, but AZT use was widespread in the study group and was not found (in a further unreported analysis) to have an influence on estimated HLA effects.

To test the effect of HLA antigens, three indicator variables were constructed for patients who were: (i) positive for the antigen or antigen combination in question; (ii) untyped; (iii) typed in insufficient detail

to judge whether they had, or had not, the antigen or antigen combination in question. Thus the baseline group was taken to be the patients who were definitely negative for the antigen in question.

Analysis of immunological progression

To see whether HLA types were associated with faster or slower immunological progression, hierarchical regression models¹⁴⁻¹⁶ were used to derive shrinkage estimates of individual rates of decay of square root CD4 count and CD4 percentage, for individuals with at least two determinations of the marker in question. The square root transformation was chosen for CD4 counts to stabilize variances and induce normality. Shrinkage estimates were used since, on average over the study group, they give considerably more accuracy than do simple least squares estimates of decay slopes.¹⁵ Shrinkage estimates are essentially very similar to the estimates derived from multilevel modelling¹⁷ which have been used to estimate root CD4 slopes in reference 18.

Associations between estimated slopes of decay and HLA antigens were sought using multiple linear regression. HLA antigens were coded using indicator variables as in the proportional hazards models (although seven were now required to analyse A1-B8-DR3 and B27 simultaneously) and additional covariates were again included to control for gender,

exposure category, age and (for the seroconversion group) method of seroconversion determination. The analysis was performed both for the whole cohort and for the seroconversion cohort, and sensitivity to the number of marker measurements used was checked and found to be modest. AZT effects were again not considered; in earlier work we found the impact of treatment to be a small and transitory increase in CD4 level without strong evidence of an accompanying alteration of slope.¹⁴

Simple plots and linear regressions of square root CD4 count against time from seroconversion were used to show graphically the longitudinal behaviour of CD4 count, as well as the timing of any clinical diagnoses, for the nine patients from the seroconversion cohort who were B27-positive; it may also be of interest to other researchers to know the full phenotypes of these patients, as far as these have been determined.

Results

Table 2 shows the numbers typed and the proportions typed positive for A1-B8-DR3 and B27. Of the 262 typed patients in the cohort, 34 had the antigens A1, B8 and DR3 in their phenotype (which is most likely to mean that they received the combination as a complete haplotype) and 13 were typed in insuffi-

cient detail to tell whether they were A1-B8-DR3 or not. Of the 155 typed patients in the seroconversion group, 21 were A1-B8-DR3 and five were not fully typed. Thus, in both cases, about 14% of the fully typed individuals were A1-B8-DR3, which was a slightly lower proportion than expected. Steel *et al.* have suggested an expected frequency of occurrence of 25% in Scotland and Jazwinska and Kilpatrick¹⁹ reported a frequency of 20% in parents of 132 babies born at an Edinburgh maternity unit (see final column of table).

Of 226 patients in the cohort fully typed for B27, 12 had the antigen B27. In the seroconversion cohort, 9/134 fully typed patients were B27. The observed frequencies of B27 in fully typed individuals (5% and 7%) were thus in line with an expected frequency of occurrence of 8%.¹⁹

Analysis of clinical progression

In the proportional hazards analysis of progression from seroconversion to clinical endpoints for the 313 members of the seroconversion cohort, A1-B8-DR3 emerged as a significant predictor of faster progression (Table 3). A1-B8-DR3 was associated with adjusted relative risks of 3.7 (95% CI 1.9–7.2) of dying, 3.1 (1.6–6.0) of developing AIDS and 1.9 (1.1–3.2) of reaching CDC stage IV. Very similar results were obtained in an analysis restricted to the

Table 2 Numbers typed positive for A1-B8-DR3 and B27

Group	HLA combination	Positive	(%)	Negative	Incomplete typing	Expected frequency ¹⁹
Whole cohort (<i>n</i> = 692, 262 typed)	A1-B8-DR3	34	14%	215	13	20%
	B27	12	5%	214	36	8%
Seroconversion (<i>n</i> = 313, 155 typed)	A1-B8-DR3	21	14%	129	5	20%
	B27	9	7%	125	21	8%

Table 3 Results of a proportional hazards analysis of the effect of A1-B8-DR3 on progression to clinical endpoints

Group	Number	Dead	RR	CI	AIDS	RR	CI	CDC IV	RR	CI
Seroconversion (<i>n</i> = 313)		82			84			185		
A1-B8-DR3	21	13	3.7	1.9–7.2	13	3.1	1.6–6.0	17	1.9	1.1–3.2
Not A1-B8-DR3	129	28	1	Baseline	27	1	Baseline	89	1	Baseline
Undetermined	5	1	1.0	0.1–7.9	1	1.0	0.1–7.0	4	3.4	1.2–9.5
Untyped	158	40	1.2	0.7–2.0	33	0.7	0.4–1.3	75	0.7	0.5–1.0
IDU subgroup (<i>n</i> = 260)		74			75			170		
A1-B8-DR3	20	13	3.8	1.9–7.5	13	3.1	1.6–6.0	17	2.0	1.2–3.4
Not A1-B8-DR3	113	27	1	Baseline	36	1	Baseline	82	1	Baseline
Undetermined	4	1	1.0	0.1–7.7	1	0.9	0.1–7.0	4	3.7	1.3–10.4
Untyped	123	31	1.2	0.7–2.0	23	0.7	0.4–1.2	59	0.7	0.5–0.9

RR, relative risk adjusted for gender, age, risk group and method of seroconversion determination.

260 members of the seroconversion cohort who were infected through IDU in the initial years of the epidemic 1983–1985 (Table 3).

The analysis also showed that possible biases induced by the nature of the typing of the cohort were not severe. The untyped patients had a significantly lowered risk of progression to CDC stage IV (RR 0.7, CI 0.5–1.0), perhaps explained by the fact that typing began with the most seriously ill patients, but this effect was not evident in the analyses to AIDS and death.

In the case of B27 the proportional hazards form of analysis was less appropriate since none of the nine patients in the seroconversion cohort who were typed positive for this antigen developed AIDS or died. However three reached CDC stage IV and a significantly reduced progression rate to this endpoint (RR 0.3, CI 0.1–0.9) could be demonstrated.

Analysis of immunological progression

Slope estimates for decay of root CD4 count could be derived for 603 members of the cohort (who possessed at least two CD4 count measurements), of whom 290 were also in the seroconversion cohort. The mean number of counts per patient was 14.9 (SD 10.4) and 491 patients (81%) had five or more counts. Estimates for rate of decay of CD4 percentage could be derived for 617 patients, of whom 295 were also in the seroconversion cohort. The mean number of measurements was 15.5 (SD 10.7) and 507 patients (82%) had more than five measurements.

In the group of 603 patients with estimated root CD4 slopes there were 33 patients with A1-B8-DR3. Their mean slope per annum was -2.1 and the standard deviation of the slopes was 1.3 . These estimates were based on a mean of 19 counts per individual (SD 9.4). In contrast, the 12 patients typed positive for B27 had a mean slope of -1.00 (SD

1.2) based on a mean of 25 counts (SD 9.3). The mean slope over the whole group of 603 was -1.6 (SD 1.0).

In linear regression analyses of root CD4 slope against HLA type (Table 4), done for both the whole cohort and the seroconversion cohort, and controlled for other covariates, there were significant associations between A1-B8-DR3 and fast immunological progression, and between B27 and slow immunological progression. Relative to the baseline group who were negative for both HLA types, the presence of A1-B8-DR3 was associated with differences in rate of decay of -0.45 (SE 0.19, $p=0.02$) for the whole cohort and -0.75 (SE 0.21, $p<0.001$) for the seroconversion cohort; the presence of B27 was associated with differences in rate of decay of 0.62 (SE 0.30, $p=0.04$) and 0.72 (SE 0.31, $p=0.02$) respectively.

Out of 617 patients assigned rates of CD4 percentage loss, the 33 patients with A1-B8-DR3 had a mean slope of -2.3 (SD 1.9), based on an average of 20 counts (SD 9.2). The 12 B27 patients had a mean slope of -0.5 (SD 1.5), based on an average of 27 counts (SD 8.9). The mean rate over the whole group of 617 was -1.7 (SD 1.4).

In linear regression analyses of CD4 percentage slope against HLA type (Table 5), done as for CD4 count, there were again significant associations between A1-B8-DR3 and fast immunological progression and between B27 and slow immunological progression. Relative to the baseline group who were negative for both HLA types, the presence of A1-B8-DR3 was associated with differences in rate of decay of -0.64 (SE 0.25, $p=0.01$) for the whole cohort and -0.70 (SE 0.24, $p<0.005$) for the seroconversion cohort; the presence of B27 was associated with differences in rate of decay of 1.18 (SE 0.40, $p<0.005$) and 1.15 (SE 0.35, $p<0.005$) respectively.

In all regression analyses, the results changed very

Table 4 Association of A1-B8-DR3 and B27 with rate of loss of square root CD4 count

Group	Whole Cohort				Seroconversion Cohort			
	<i>n</i>	Coeff.	SE	<i>p</i>	<i>n</i>	Coeff.	SE	<i>p</i>
	603				290			
A1-B8-DR3	33	-0.45	0.19	0.02	21	-0.75	0.21	<0.01
Perhaps A1-B8-DR3	5	-0.13	0.46	0.78	2	-0.10	0.64	0.87
B27	12	0.62	0.30	0.04	9	0.72	0.31	0.02
Perhaps B27	27	0.24	0.21	0.24	18	0.33	0.23	0.15
Perhaps either	8	-0.05	0.37	0.89	3	0.53	0.52	0.31
Untyped	344	0.06	0.10	0.53	135	0.05	0.12	0.66
Neither A1-B8-DR3/B27	173	Baseline				Baseline		

Multiple linear regression analyses controlled for sex, exposure category, age and method of seroconversion determination (for the seroconversion cohort).

Table 5 Association of A1-B8-DR3 and B27 with rate of loss of CD4 percentage

Group	Whole cohort				Seroconversion cohort			
	<i>n</i>	Coeff.	SE	<i>p</i>	<i>n</i>	Coeff.	SE	<i>p</i>
	617				295			
A1-B8-DR3	33	-0.64	0.25	0.01	21	-0.70	0.24	<0.01
Perhaps A1-B8-DR3	5	-0.68	0.60	0.26	2	-1.01	0.72	0.16
B27	12	1.18	0.40	<0.01	9	1.15	0.35	<0.01
Perhaps B27	28	0.52	0.27	0.06	18	0.52	0.26	0.05
Perhaps either	8	0.19	0.48	0.70	3	0.25	0.60	0.68
Untyped	358	0.10	0.13	0.44	135	0.04	0.13	0.77
Neither A1-B8-DR3/B27	173	Baseline				Baseline		

Multiple linear regression analyses controlled for sex, exposure category, age and method of seroconversion determination (for the seroconversion cohort).

little when the selection criteria were changed so that only patients with five or more marker determinations were eligible. A1-B8-DR3 remained associated with faster progression and B27 with slower.

When the 603 patients with estimated root CD4 slopes were ranked by these slopes and divided into quarters representing very fast, moderately fast, moderately slow and very slow progressors (Table 6) 13/33 A1-B8-DR3 carriers (39%) were in the fastest quarter and 13 in the intermediate half; however, seven (21%) were found in the slowest quarter, showing that A1-B8-DR3 does not lead exclusively to poor outcomes. However, among the 13 patients in the fastest quarter there were some exceptionally fast progressors: seven (21% of 33) were in the fastest 10% of the group, and five (15% of 33) were in the fastest 5% of the group.

Seven out of 12 B27 patients (58%) were in the slowest quarter, three were in the intermediate half and two (17%) were in the fastest quarter. Plots of the nine B27 patients from the seroconversion cohort mostly showed slow CD4 loss or stable counts (see Figure 1), with the exception of patient 209901 who was one of the two patients in the fastest quarter. Simple linear regression lines indicated that no patient had fallen below a CD4 count threshold of 100/mm³ within 10 years and there were no AIDS

diagnoses or deaths. Three of these patients had been diagnosed with ARC, as had the three other B27 patients who were not members of the seroconversion cohort; one of these also reached AIDS although none had died.

Discussion

Our results support previous studies which have shown the haplotype A1-B8-DR3 to be associated with fast progression of HIV disease.¹⁻³ We have conducted a much larger study of the influence of HLA antigens in the setting of a prospectively followed cohort where accurately estimated seroconversion times are available for a sizeable subgroup. Our data gave strong evidence of faster average clinical and immunological progression in patients carrying this haplotype and, given this rapidity of progression, they raise the issue of whether HLA type should be considered in the context of future prospective clinical trials.

The City Hospital cohort from which these data come is dominated by a young group of needle-sharing IDUs who were mostly infected in 1983-1985.⁶⁻¹⁰ Some 470 (68%) cohort members and 281 (90%) seroconversion cohort members are believed to have been infected through IDU, 260 of

Table 6 Rates of CD4 loss in 603 patients ranked into quarters and crosstabulated with HLA

Group	Fastest quarter (<i>n</i> = 151)	Intermediate (<i>n</i> = 151)	Intermediate (<i>n</i> = 151)	Slowest quarter (<i>n</i> = 150)
A1-B8-DR3	13	8	5	7
Perhaps A1-B8-DR3	2	0	3	0
B27	2	0	3	7
Perhaps B27	6	5	8	9
Perhaps either	3	0	3	2
Neither	67	25	31	59
Untyped	58	113	98	66

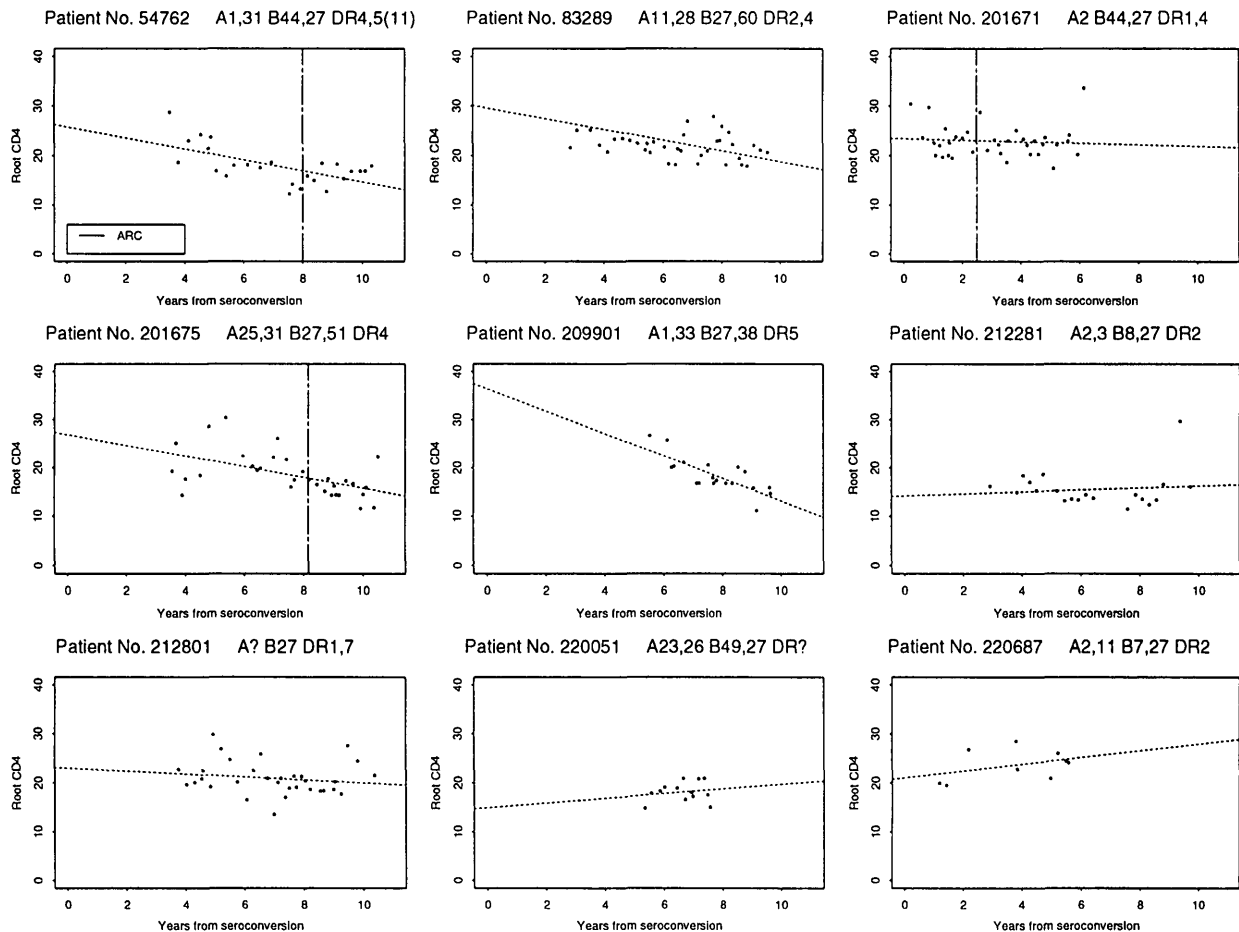


Figure 1. CD4 decline on square-root scale for nine B27 patients with well-estimated seroconversions; dotted lines are linear regression estimates of the patterns of root CD4 decay; vertical lines denote CDC stage IV diagnoses (marked ARC); full HLA types are given.

the latter in 1983–85. To maximize numbers we considered all patients in this paper, regardless of risk group. In Table 2, in an analysis of clinical progression restricted to the 260 IDUs infected in 1983–85, we checked that very similar A1-B8-DR3 results were obtained to those derived from the whole seroconversion cohort.

We were able to look carefully at HLA effects on clinical progression because of our accurate knowledge of seroconversion times for a subset (45%) of the cohort. In seroconversions estimated from interval data (85% of cases) the maximum possible error in the estimate was one year; in the remaining cases estimated through observation of seroconversion illness and detailed knowledge of drug-using behaviour, the estimates were also likely to be accurate. It was possible to make accurate interval seroconversion estimates for so many people because of the availability of stored sera.

Because of our knowledge of seroconversions, we could use survival analysis methods based on the proportional hazards model, with survival measured from this natural origin. This is the most accurate

way of estimating the influence of HLA antigens, or any other covariates, on clinical progression. Studies which measure progression times from other origins such as first known occasion of HIV-positivity (so-called prevalent cohort studies such as the study of Kaplan *et al.*³ are prone to a series of biases in estimation of covariate effects, especially onset confounding.²⁰ Studies which use case-control methods (such as those of Steel *et al.* and Kaslow *et al.*^{1,2} cannot estimate the relative progression risks associated with HLA type.

The second strength of our study was the availability of a large amount of longitudinal CD4 count and CD4 percentage data which allowed the study of HLA associations with immunological progression. CD4 monitoring is performed regularly in the Edinburgh cohort (every three months for most patients, more frequently for the sicker patients and patients on AZT) and at 24 March 1994, the mean number of counts per patient was 13.1 over the whole cohort; 491 patients (71%) had five or more counts. With this volume of data, the characterization of decay rates in the cohort becomes possible but,

because CD4 count and CD4 percentage are volatile markers and because the amount and quality of the information on decay rate vary from individual to individual, the use of hierarchical-model-based shrinkage methods is preferable to the application of simple maximum likelihood (least squares) estimation.¹⁵ Our slope estimates are thus likely to be more accurate than those used in earlier A1-B8-DR3 studies,¹⁻³ and the number of patients for whom we have slope estimates is much greater.

We chose to use all possible available information on rate by including patients with as few as two CD4 counts in our analysis. This had negligible bearing on our study of HLA associations with rate (Tables 3 and 4) since (a) estimates for patients with few counts are generally set very close to the mean rate and are not influential, and (b) patients with types A1-B8-DR3 and B27 generally had more markers measurements than average. However, we also verified empirically that very similar results were obtained when the analysis was restricted to patients with at least five counts.

The prevalence (14%) of A1-B8-DR3 in the fully typed individuals in this study was slightly lower than expected (20%). It is possible that a larger proportion of those who died rapidly before typing began carried the haplotype, although this hypothesis is now difficult to test. It seems less likely that additional members of the cohort died rapidly with B27, since this HLA antigen is rare and the observed frequencies matched the expected frequencies.

On the other hand, for the IDU members of the study group, it could also be argued that the expected population frequencies are not appropriate comparisons, since the study group is not a completely random sample from the general population and there were many needle-sharing networks which included several family members. Because of the need to preserve confidentiality, it is difficult to initiate an active study of familial links in the cohort but, in an informal enumeration by City Hospital clinicians, it was found that among 164 IDU patients with at least ten CD4 determinations it was possible to count 17 patients in eight families with first-order relationships (sibling, parent-child) to other cohort members, and this was merely a lower bound on the total number of blood relationships in this group where there are also second-order relationships (cousin, uncle/aunt).

The underlying biological basis for the association of A1-B8-DR3 with rapid progression of HIV-related disease and CD4 loss remains obscure. The haplotype A1-B8-DR3 is well known to be associated with a wide range of autoimmune disorders and individuals bearing this haplotype could be considered to be immunologically 'hyper-responsive' (see also discussion of Steel *et al.*¹ This suggests that autoim-

munity may be responsible for the progressive immunodeficiency rather than any direct cytopathic effect of HIV on CD4 lymphocytes, but the mechanism at the molecular level by which this occurs is still unclear.

The second major association we observed was between B27 and slow progression. One of 12 patients typed positive for this antigen reached AIDS, but no patients died, and it seemed that these patients were experiencing slow immunological decay. There has been recent interest in the phenomenon of long-term survival in HIV patients and in the explanation for the differences between such patients and rapid progressors.^{21,22} One suggestion put forward is that differences are due to a less virulent strain of HIV-1²² but our data, as well as those of others, raise the possibility that it could also be due to decreased susceptibility in the host. In two recent reports the number of long-term survivors (10–15 years) infection varied from 5 to 15% of cohorts.^{23,24} One of these cohorts reported on HLA associations, and noted greater frequencies of A32, B4 and C2 in the long-term survivors.²³ Our report of an association with B27 adds further to the suggestion that there may be an immunological as well as, or instead of, a virological explanation of the phenomenon of long-term survival.

In addition, there appears to be an underlying molecular basis for our observation of slow progression in patients with B27. The experiments of Ohno⁴ have shown that the nonapeptide derived from gp120 has virtually 'optimal' binding characteristics for HLA B27 and is therefore likely to be 'tolerogenic' in that it resembles the preferred self peptides presented to T cells on this molecule. It could therefore be argued that this association of B27 with a slow immunological progression supports the proposal that the immunodeficiency that develops following HIV infection is due to an autoimmune mechanism.

Habeshaw has proposed that the gp120 carboxy-terminal region mimics HLA DR beta chain alleles for class 1 presentation. In the case of DR B1 alleles presented by HLA B27, the binding motif expressed by the DR B1 chain is well mimicked by the carboxyterminal gp120 sequence. For the DR B3 alleles, the mimicry is poor and so the HIV-1 carboxyterminal sequence would represent an allo-epitope of DR B3 when expressed upon the B27 molecule. The presence of an allogeneic MHC component (or peptide mimic thereof) 'breaks tolerance' of self MHC-derived peptides.²⁵⁻²⁷

In summary, we have confirmed that A1-B8-DR3 is associated with a rapid development of immunodeficiency after HIV infection. However we have also observed that HLA B27 is associated with a slow development of HIV immunodeficiency, a finding that could be expected if autoimmune mechan-

isms played a major role in the development of immunodeficiency, since studies at the molecular level have demonstrated that a nonapeptide derived from the HIV envelope protein is tolerogenic when presented to T cells on the HLA B27 molecule.

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